Communications in Applied Sciences
ISSN 2201-7372
Volume 2, Number 1, 2014, 52-64

Anti Bacterial Activity of Ethanolic Extract of *Zingiber Officinale* and *Pipper Nigrum* against Some Clinical Isolates

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Abstract. Ethanolic extract of *Zingiber officinale* and *Pipper nigrum* were evaluated by testing in vitro antimicrobial activity on clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, and *proteus specie* using Agar diffusion method. *Proteus specie* showed sensitivity to *Z. officinale* at disc concentration range of 50 to 800µg/ml, *Staphylococcus aureus* also showed sensitivity to *z. officinale* at concentration of 100 to 800 µg/ml and *Klebsiella pneumonia* showed sensitivity at disc concentration of 100 to 800 µg/ml likewise proteus specie showed sensitivity to *p. nigrum* at disc concentration range of 100 to 800µg/ml and *staphylococcus aureus* also showed sensitivity to *p. nigrum* at disc concentration range of 100 to 800µg/ml. However, the Ethanolic extract of *z. officinale* and *p. nigrum* showed no lethal or inhibitory effects on *Pseudomonas aeruginosa* and *Escherichia coli* at all concentration. The observed minimum inhibitory concentration (MIC) of the Ethanolic extraction for *proteus species*, *Staphylococcus aureus* and *Klebsiella pneumonia* were 50, 100,200µglml respectively for *Z. officinale*, while for *P. nigrum*, the minimum inhibitory concentration (MIC) were 100 and 200µg/ml for Proteus specie and staphylococcus aureus respectively. The extracts were further subjected to phytochemical tests for the presence of secondary metabolites using standard procedures. The results of phytochemical screening indicated the presence of alkaloids, Flavonoid, reducing sugar, saponins and steroids in Z. officinale, and alkaloids, Flavonoid, saponins, steroids and tannin in *P. nigrum*. This indicates that both *Z. officinale* and *piper nigrum* has the potential for the production of drugs against pathogenic organisms.

KEY WORDS: Antibacterial, Ethanolic, *Zingiber Officinale, Pipper Nigrum* andClinical Isolates.

Introduction

Medicinal plants are cheap and renewable sources of pharmacologically active substances and are known to produce certain chemicals that are naturally toxic to bacteria (Basile *et al*, 1999). The use of medicinal plants to treat aliment associated with pains is well known through history (Ernest and Pittler, 2000). Such plant can play important role in drug discovery is logical research strategies in the search for new drugs. In Nigeria and Africa in general, the use of herbs and medicinal plants for therapy is common practice (Soforowa; 1993). A medicinal plant therefore, is describe as any plant which is one or more its organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Soforowa; 1993). Ginger is a herb. The rhizome (underground stem) is used as a spice and also as a medicine. It can be used fresh, dried and powdered, or as a juice or oil. *Zingiber officinale* has been used as a medicinal plant in Asia, India, Jamaica and Nigeria. In China, ginger has been used to aid digestion, treat stomach upset, diarrhea and nausea for 2000 years (Muaz, 1999, Azu and Onyeagba 2007). Ginger has a wide range of action on the human body and has been found effective in the treatment of cataract, heart disease, migraines, struck amenorrhea, athletes foot, bursitis, chronic fatigue, cold, flu, coughs, depression, dizziness, fever, erectile difficulties, kidney stones, renal disease and viral infection (Peggy, 2006). It is a valued remedy for coughs and bronchitis and also serves as a soporific in fever its natural diuretic stimulates the kidney to flush out toxins faster. In Panama, it is used to relieve rheumatism. In Guatemala and Trinidad, it is the best remedy for stomach ache, malaria, indigestion the fumes from an infusion in urine are inhaled to relieve head colds, due to prevalent resistance of microorganism to drugs and other therapeutic agent. *Pipper nigrum* (Black pepper) is used as a traditional medicine for the treatment of both Gram positive and Gram negative bacteria in China, India, Asia etc (Gong *et al,* 1985). Black pepper also stimulates bite secretion without increasing the volume of cholesterol and bile acid (Genesh *et al* ,1987).

MATERIALS AND METHODS

Sample collection

The test organisms which are isolated from patients were collected from Aminu Kano Teaching Hospital. The test organisms used for this research includes;

- *Staphylococcus aureus*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Klebsiella pneumonia*
- *Proteus specie*

Collection of Plant Materials

The plant materials of *zingiber officinale* (Ginger) and *Piper nigrum* (Black pepper) used for the study were purchased from Kurmi market in Dala Local Government Area of Kano State. The plant was authenticated in the Department of Biological Science, Bayero University, Kano by Prof. B. S. Aliyu..

Extraction of Plant Materials

Fresh rhizome of *Zingiber officinale* (Ginger) and *Piper nigrum* (Black pepper), were obtained and washed with distilled water and allowed to dry (air dry) for two days and macerated with clean pestle and mortar in the laboratory as described by Muktar and Tukur (1999). Extraction was done using extraction. Twenty grams (20grms) of each macerated materials were percolated at room temperature with 250ml each of absolute ethanol (99%) in a liter each of conical flask. The flask were corked, shaken and left to stand for two (2) weeks with constant shaking at regular intervals (Fatope *et al,* 1993).

The crude extracts of *the Z. officinale* and *P. nigrum* obtained were carefully labeled and weighed in a refrigerator at 4^{o}c for further analysis.

Phytochemical Screening

Test for alkaloids

To 0.1ml of the extract and fractions in a test tube, 2-3 drops of Dragendoff's reagent was added. An orange red precipitate with turbidity denoted the presence of alkaloids (Clulci, 1994).

Test for Flavonoid

To 4mg/ml of the extracts and fractions a piece of magnesium ribbon was added followed by drop-wise addition of concentrated HCI. A colour change from orange to red indicated the presence of flavones; red to crimson indicated presence of Flavonoid (Sofowora, 1993).

Test for glycosides

Ten milliters of 50%H_2SO_4 was added to 1ml of the filtrate in separate test tubes and the mixtures heated for 15mins followed by addition of 10ml of Fehiling's solution and boiled. A brick red precipitate indicated presence of glycosides (Soforowa, 1993).

Test for reducing sugars

To 1ml of extract and fraction in separate test tubes, 2.0mls of distilled water were added followed by addition of Fehiling's solution (A+B) and the mixtures were warmed at 40^{o}C. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar (Brain and Turmer, 1975).

Test for saponins

Half gram of the powdered leaf was dispensed in a test-tube and 5.05ml of distilled water was added and shaken vigorously. A persistent froth that lasted for about 15 minutes indicated the presence of saponins (Brain and Turner, 1975).

Test for steroids

Two millitters of the extracts were evaporated to dryness in separate test tubes and the residues dissolved in acetic anhydride followed by addition of chloroform. Concentrated sulphuric acid was added by means of a pipette via the side of the test tubes. Formation of brown ring at the interface of the two liquids and violet color in the supernatant layer denoted the presence of steroids (Clulci, 1994).

Test for Tannins

Two milliliters of the extract/fraction was diluted with distilled water in separate test tubes, 2-3 drop of 5% ferric chloride (FeCl3) solution was added. A green-black or blue colouration indicated tannin (Clulci, 1994).

Inoculums Standardization

Inoculums were prepared by direct colony suspension where 3-4mls of sterile physiological saline was poured into a test tube for which a loopful of the colonies of the test isolate taken directly from the plate was emulsified and the suspension adjusted to match with that of 0.5 McFarland standard which has similar appearance of an overnight broth culture by adding distilled water (Azu and Onyeagba 2007, Garc and Bruc 1993,Cheesebrough,2000)).

Culture Medium

The culture medium used for sensitivity testing was nutrient agar (NA). these were prepared according to manufacturers specifications and guidelines.

Preparation of Sensitivity Disc

Disc were punched using No.1 Whitman filter paper with the diameter of 6mm, and were sterilized by dry heat at 140^{o}c for 1 hour. The disc were allowed to cool, using screw-capped bottle, different concentrations of the plant extract (*Z. officinale* and *P. nigrum*) were prepared using Dimethyl Sulphur Oxide (DMSO) to each of the different weight of the extract which arrived at the concentration ofm50, 100, 200, 400, and 800µg/ml. 50 pieces of the paper disc were introduced into 0.5ml of the different concentration of extracts and allowed to stand until the whole concentration was completely absorbed by the filtration disc, because each disc is capable of absorbing 0.01ml (Kirby-Beuer, 1960).

Sensitivity Test

The Agar diffusion method as described by Deeni and Hussein (1971) was adopted in the sensitivity of the test organisms to the extract of *zingiber officinale* (ginger) and *Piper nigrum,* nutrients agar was used as the medium in 100ml disposable petridish. The prepared plates were dried in a drier to remove excess surface moisture. 0.021ml of the suspension was added to the medium using a glass dropper and a sterile swab stick was used to spread by streaking the organisms allover the surface of the medium and allow to dry for 5min. cups of 6mm is diameter was made in the agar using sterile cork borer.

Impregnated sterile paper disc of Whitman No.1 filter paper containing the crude extracts of *Z. officinale* and *p. nigrum* at a concentration of 50,100,200,400, 800µg/ml were arranged two disc per plate and standard antibiotic (Augmenting 30µg) was placed on the surface of the inoculated media and were pressed firmly to ensure even contact. The disc were sufficiently spaced out to prevent overlapping of zones. The plate were inverted and allowed to stand for 30mins for the extract to diffuse into the agar. The process was repeated in triplicate plates and all the plates were incubated aerobically at 37°C for over night. (Kirby-Baver 1966 and Mukhtar and (Tukur: 2000).

Measurement of Zone of Diameter

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of the extract were prepared by serial doubling dilution using distilled water to obtain concentrations of 50µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, 800 µg/ml. equal volume (2mls) and Nutrient broth were mixed. Specifically 0.1ml of standardized inoculate ($3.3x16^6$; CFU/ml) was added to each of the test tubes above. The tubes were incubated aerobically at 35°c for 24 hours. Tubes containing broth and extracts without inoculate which served as positive control while tubes containing broth and inoculate served as negative control. The tubes were observed after 24 hours of incubation to determine minimum inhibitory concentration. That is the lowest concentration that showed no evidence of growth (Akinyemi *et al.,* 2005; Vallekobia *et al.*, 2001).

Results

Table 1: Weight of Extract Recovered and their Physical Appearance

Extract	Weight of extract recovered	Physical appearance of the extract		
		Colour	Texture	Odour
Zinginber officinale (Ginger)	2.60	Brown	Gummy	Pungent
Piper nigrum (Black pepper)	3.50	Dark brown	Oily	Pungent

Table 2: Antimicrobial activity of Ethanolic extract of *zingber officinale*

	Concentration of Extract (µg/m)					
	Zone of Inhibition (MM)					
Test organisms	Control	50	100	200	400	800
Staphylococcus aureus	00	00	09	12	17	20
Pseudomonas aeruginosa	00	00	00	00	00	00
Klebsiella pneumoniae	00	06	06	10	15	18
Escherichia coli	00	00	00	00	00	00
Proteus specie	00	10	13	14	18	19

Table 3 Minimum of inhibitory concentration (MIC)

Test Organisms	MIC (µg/ml)
Staphylococcus aureus	100
Pseudomonas aeruginosa	-
Klebsiella pneumoniae	200
Proteus specie	50

Table 4: Antimicrobial activity of Ethanolic extract of *Piper nigrum*

	Concentration of Extract (µg/m)					
	Zone of Inhibition (MM)					
Test organisms	Control	50	100	200	400	800
Staphylococcus aureus	00	00	08	08	09	12
Pseudomonas aeruginosa	00	00	00	00	00	00
Klebsiella pneumoniae	00	00	00	00	00	00
Escherichia coli	00	00	00	00	00	00
Proteus specie	00	00	11	13	15	18

Table 5 Minimum of inhibitory concentration (MIC) of Ethanolic extract of *Piper nigrum*

Test Organisms	MIC (µg/ml)
Staphylococcus aureus	200
Pseudomonas aeruginosa	-
Klebsiella pneumoniae	-
Escherichia coli	-
Proteus species	100

Table 6 Result of Phytochemical Screening of *Zingiber Officinale* and *Pipper nigrum*

Extract	Alkaloids	Flavonoid	Glycosides	Reducing sugar	Saponins	Steroids	Tannin
z. .officinale (Ginger)	+	+	-	+	+	+	-
P. nigrum (black pepper)	+	+	-	-	+	+	+

Key + = Present
\- = Absent

Discussion

The results from the Ethanolic extracts of both *Zingiber officinale* and *Piper nigrum* posses antimicrobial activity on three (3) clinical isolates namely *Staphylococcus aureus*, *Klebsiella peumoniae*, and Proteus specie. That the Ethanolic extract of *Z. officinale* is more effective on *Staphylococcus aureus* and *Proteus specie* then followed by *Klebsiella peumoniae*. But the Ethanolic extract of *P. nigrum* is active on *Proteus specie* and then followed by *Staphylococcus aureus*. However, *Z. officinale* and *P. nigrum* extract used in this study did not demonstrate any

inhibitory activity on *Escherichia coli* and *Pseudomonas aeruginosa*. This finding concurred with that of Thannivanvara *et al,* (1997), in which this plants extract did not shows any inhibitory activity against *Escherichia coli* and *Pseudomonas aeruginosa* at variable concentrations. The insensitivity of *Escherichia coli* and *Pseudomonas aeruginosa* to the extract of *Z. officinale* and *P. nigrum* were demonstrated by Misas *et al*, (1997) using 95% ethanol. But in another work done by Misas *et al*, (1997) acetone extract of *Z. officinale* and *P. nigrum* shows inhibitory against *Escherichia coli* and *Pseudomonas aeruginosa*. However, Moscola *et a*l, (1989) demonstrated the inhibitory activity of the extract of *Z. officinale* and *P. nigrum* on *Proteus specie* and *Staphylococcus aureus*. Thus, finding concurred with our findings. Nakamura *et al,* (1999) reported that activity of both *Z. officinale* and *P. nigrum* were used traditionally in Brazilian folk medicine to treat different disease e.g. upper respiratory tract, gastrointestinal haemorrghea, nausea, vomiting as well as cough. Rakamans *et al,* (2000) stated that *P. nigrum* shows activity on *Proteus specie*, moderately active on *Pseudomonas aeruginosa* and weakly active against *Escherichia coli*. It has been reported that *Z. officinale* extract have antibacterial effects against both Gram positive and Gram negative bacteria such as *Staphylococcus aureus* but inactive on the *Pseudomonas aeruginosa* (Moscola *et al,* 1989). The insensitivity exhibited by *Pseudomonas aeruginosa* to *Z. officinale* in this study and the non susceptibility of *Pseudomonas aeruginosa* to herbal extract in most investigation such as those of Indo , (1982), Mukhtar and Shu'aibu (1999), Mukhtar and Tukur; (2000) and Mukhtar and Okafor; (2001) ascertain that *Pseudomonas aeruginosa* is inherently resistance to many antibiotics and can mutate to even more resistant strain during therapy (Murry *et al,* 1998). However, according to Barley and Scott; (1974) in vitro reaction does not always give the extract or similar results when compared to in vivo test. In essence, the observed potency of an antimicrobial in vitro might not be obvious when the same antimicrobial is subjected on in vivo test. And as such results from an in vitro test like the one obtained from this study might not necessary represent what might be obtainable from an in vivo study. A Chinese case series reported that an herbal

mixture containing *Z. officinale* and *P. nigrum* were effective in halting upper gastrointestinal haemorrghea (Gong *et al,* 1985). There is no report of bleeding problems in person consuming up to 5grms of dried Ginger (Lumb; 1994). Data on *Z. officinale*'s effectiveness in preventing post operative nausea complication. In two randomized, double blind studied of woman undergoing Gynecological survey, these treated with Ginger had significantly less post -operative nausea and vomiting than those treated with placebo (Visalyaputra *el al,* 1998). Likewise, the results obtained shows that the minimum inhibitory concentration (MIC) of *Z. officinale* and *P. nigrum* against *Proteus specie* were 50 and 100ug/ml respectively, this agree with that obtained by Chen *et al,* (1987). It was also stated by Chen *et al,* (1987) that the minimum inhibitory concentration (MIC) of *Z. officinale* and *P. nigrum* against *Staphylococcus aureus* were 100 and 200ug/ml respectively. The result of phytochemical screening of Ethanolic extracts of *zinginber officinale* revealed the presence of alkaloids, flavonoids, reducing sugar, saponin and steroids. *Also p. nigrum* revealed the presence of alkaloids, flavonoids, saponins, steroids and tanning. These metabolites have been reported to posses antimicrobial activity. (Cowan, 1999). In particular the flavonoids were reported to be responsible for ethno medicinal plants (Singh and Bhat, 2003).

Conclusion:

The results obtained also suggest good potency or high concentration of active compound in the extracts. Since the extracts are active against *Staphylococcus aureus,* which is Gram-positive bacterium, *Klebsiella pneumonia* and *Proteus specie*, which are Gram negative bacteria and it may not be incorrect to say that the plant is broad-spectrum antibacterial agent. As such the plant can be utilized to argument the service of primary health care.

Recommendation

Having evaluated the antimicrobial activity of the plants and found to be potent on some strain of microorganisms used, the Pharmaceutical Research Institution " and Pharmaceutical Industries in Nigeria should improve to purify and develop these plant extracts since its highly potent; economically dependent, cost efficient broad

spectrum antimicrobial (Drug) instead of relying on expensive and mostly foreign brands of antibiotics dispensed in a developing countries like ours. The plant has also reported to have antiviral, antifungal and anti yeast as such there is need for further research. Attempt should also be made to isolate and characterize the pure organic compound constituting the active secondary metabolites found in the plants so as to estimate its chemotherapeutic value, which may lead to its spontaneous use as a potent antimicrobial drug.

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